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CASE REPORT

Hypopigmented mycosis fungoides: An immunological investigation of tumor-infiltrating T cells

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ABSTRACT

Hypopigmented mycosis fungoides (HMF) is a clinical variant of MF presenting a good prognosis. In this report, we describe a case of HMF with dense infiltration of tumor-infiltrating leukocytes that bear several cytotoxic molecules, such as granulysin and T-cell intracellular antigen-1. In addition, our immunohistochemical study revealed that the ratio of $Foxp3^+$ regulatory T cells to the total $CD4^+$ cells is decreased in the lesional skin of HMF, compared with that of conventional MF. Our case suggested possible mechanisms for the hypopigmentation and good prognosis of this type of MF.

Introduction

Hypopigmented mycosis fungoides (HMF) is described as a clinical variant of MF presenting a good prognosis,[1,2] but the condition is not clearly defined by the World Health Organization or the European Organization for Research and Treatment of Cancer classification of cutaneous lymphoma.[3] Recently, Boulos et al reported a case series of 34 patients with juvenile-onset mycosis fungoides,[1] which suggested that 53% of juvenile-onset MF is a hypopigmented type, and about 40% of them contained $CD8^+$ immunophenotypes.[1] Notably, the infiltration of CD8 determines the prognosis of several types of lymphomas, which suggested possible reasons for the good prognosis of HMF.[4,5]

Regulatory T cells (Tregs) are classically identified by the expression of CD4, CD25, and Foxp3.[6] Tregs induce immunological tolerance in cancer patients by a variety of mechanisms together with other suppressor cells.[7] For instance, depletion of $CD4^+CD25^+$ Tregs enhances the immune responses against tumor antigens to induce an antitumor immune response in melanoma patients.[8] In the dermatopathological field, we previously reported significant difference in Tregs between invasive and noninvasive skin tumors, such as extramammary Paget's disease (EMPD), squamous-cell carcinoma, and Bowen disease.[9–11] These reports suggested that the ratio of Tregs could be connected with the cancer progression in skin tumors. In this report, we describe a case of HMF, and investigate the profiles of tumor-infiltrating leukocytes (TILs) in the lesional skin of this patient.

Case Report

A 22-year-old Japanese woman visited our outpatient clinic with an 11-year history of multiple vitiligo. She had been treated with topical 0.1% tacrolimus for 9 years for vitiligo vulgaris in another clinic. On her initial visit, physical examination revealed multiple vitiligo with central faint erythema on the lateral side of her trunk and lower limbs (Figure 1A). A biopsy specimen showed that atypical large lymphocytes densely infiltrated mainly the upper dermis region with involvement of the overlying epidermis (Figure 1B). Immunohistochemical staining revealed that these atypical lymphocytes, which were distributed from the upper layer of stratum spinosum to the dermis, were negative for CD4 (Figure 1C) and CD7 (Figure 1D), and positive for CD3, CD5, and CD8 (Figure 1E). There were few $CD161^+$ cells in the dermis. To further confirm the profiles of TILs in MF, we employed double staining for CD8 with granulysin (Figure 2A) or CD8 with CD7 (Figure 2B), which revealed that $CD8^+$granulysin$^+$ cells and $CD8^+CD7^+$ cells were distributed at the basal layers of the epidermis and the

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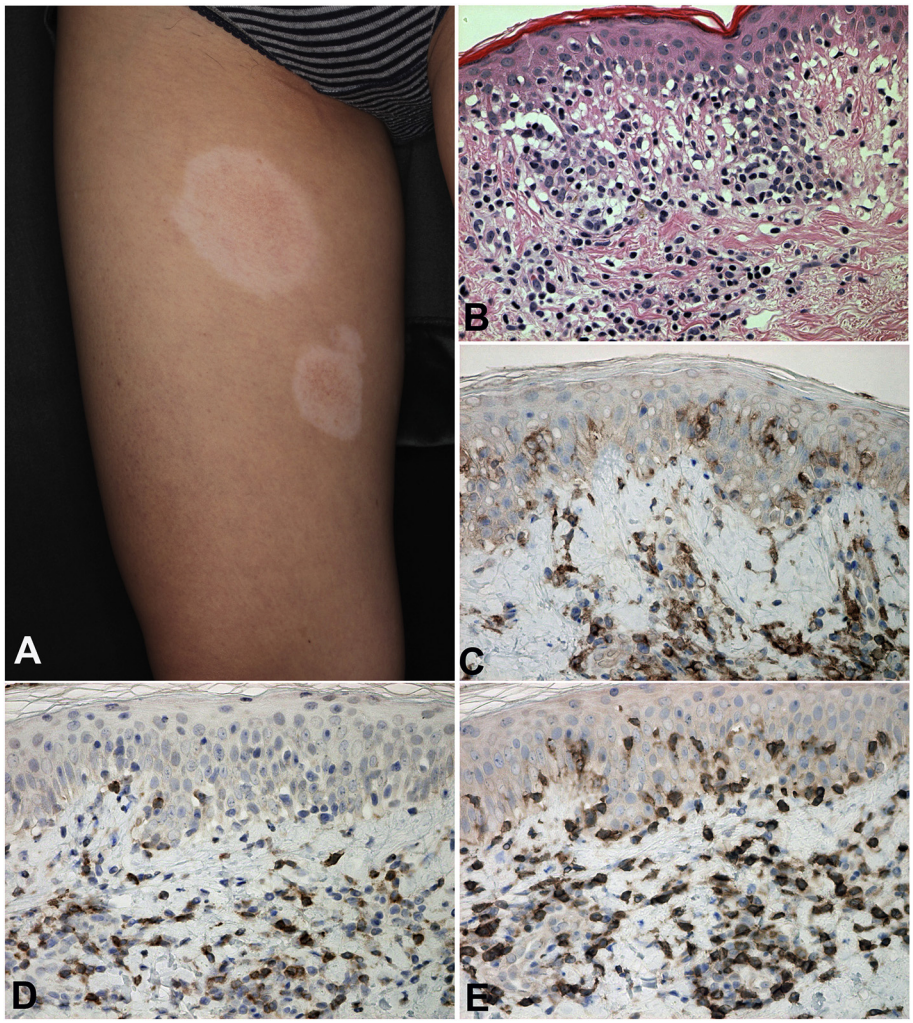

Figure 1 (A) Multiple vitiligo with central faint erythema on the lower limbs. (B) Atypical large lymphocytes can be seen densely infiltrating mainly the upper dermis with involvement of the overlying epidermis. Paraffin-embedded tissue samples were deparaffinized and stained with (C) anti-CD4Ab, (D) anti-CD7Ab, or (E) anti-CD8Ab. The sections were developed with 3,3′-diaminobenzidine tetrahydrochloride (C) (original magnification 200×). Ab = antibody.

dermis, whereas $CD8^+CD7^-$ cells and $CD8^+$ granulysin$^-$ cells were distributed from the upper layer of stratum spinosum to the dermis. Substantial numbers of T-cell intracellular antigen-1 (TIA-1)$^+$ cells were also detected in the area of distribution of TILs (data not shown). The double staining of Foxp3 with CD4, CD8, or CD25 revealed that substantial numbers of $CD4^+Foxp3^+$ cells and $CD25^+Foxp3^+$ cells were detected in the dermis (Figure 2C). There is no $CD8^+Foxp3^+$ cell in the lesional skin of HMF, as we previously reported (data not shown).[10] The percentage of $Foxp3^+$ cells was 18.4% ± 6.7% and 16.7% ± 6.3% among $CD4^+$ cells and CD25 cells, respectively. We summarized the ratio of $Foxp3^+$ cells in our case and compared it with the five cases of conventional MF, five cases of noninvasive EMPD, and invasive EMPD cases, as we previously reported (Figure S1).[10,12] We treated our patient with narrow-band UVB five times a week for 4 weeks; most lesions, however, repigmented 2 months after the treatment.

Discussion

HMF is described as a juvenile type of MF that has a relatively good prognosis compared with conventional MF.[1,2] Although the prognosis of HMF is relatively good, and the profiles of TILs determine the prognosis of cutaneous T-cell lymphoma (CTCL),[4,5,13] the precise immunological profiles of HMF have not been reported yet. Therefore, in this report, we shed light on the profiles of T cells infiltrating around the tumor cells, particularly focusing on Tregs and cytotoxic molecules.

To investigate the Tregs in HMF, we employed double staining for CD4/Foxp3 and CD25/Foxp3, which revealed a decrease in the ratio of $Foxp3^+$ on CD4 cells and $CD25^+$ cells in the lesional dermis of HMF compared with conventional MF. As we previously reported, the ratio of $Foxp3^+$ Tregs in MF is approximately 30% in the dermis, which is much higher than in skin inflammatory disorders, such as psoriasis (5%) and eczematous dermatitis (15%).[12] Compared with conventional MF, the ratio of $Foxp3^+$ Tregs in our case was decreased. Notably, a recent report suggested that Tregs play a pivotal role in maintaining peripheral tolerance that actively suppresses effector T cells.[7] In the tumor site, in concert with tumor-associated macrophages, Tregs maintain the immunosuppressive microenvironment and promote tumor growth.[7] For example, Mahnke et al[8] reported that the depletion of Tregs in melanoma patients *in vivo* resulted in enhanced immune functions and the substantial development of antigen-specific $CD8^+$ T cells in vaccinated individuals. Ni et al[5] reported that the reduction of Tregs

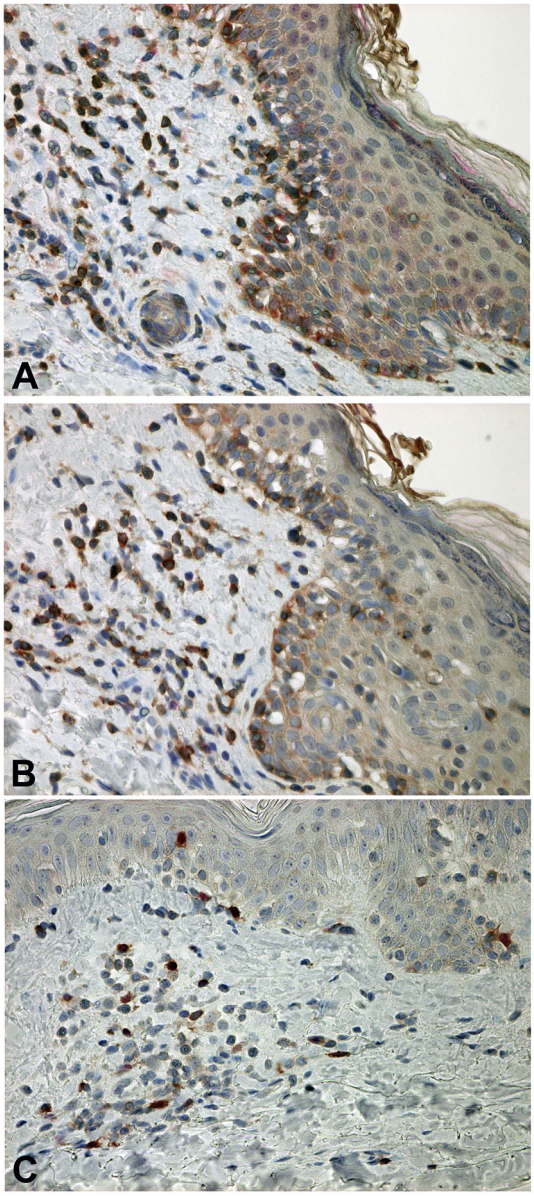

Figure 2 Paraffin-embedded tissue samples were deparaffinized and double stained with (A) anti-CD8Ab (brown) with antigranulysin Ab (red), (B) anti-CD8Ab (brown) with anti-CD7Ab (red), or (C) anti-CD25Ab (brown) with anti-Foxp3Ab (red). The sections were developed with liquid permanent red and 3,3′-diaminobenzidine tetrahydrochloride (original magnification: 200×). Ab = antibody.

by mogamulizumab leads to the increase of $CD8^+$ T cells and improves the prognosis in patients with CTCL. These reports suggested that the reduction of Tregs contributes to the increase in the number of $CD8^+$ cells and natural killer cells in patients with MF,[5] which might determine the therapeutic effects of mogamulizumab for MF. Indeed, in contrast to the decrease of $Foxp3^+$ Tregs in our case, substantial numbers of $CD7^+CD8^+$granulysin$^+$ cells were detected.

Among the cytotoxic molecules, we investigated granulysin and TIA-1 in this case. Recently, the expression of granulysin has been reported to determine the prognosis of several types of lymphomas.[4,13,14] Another cytotoxic molecule, TIA-1 in the TILs, is also reported to correlate with the therapeutic effect of several reagents for various types of cancer, including lymphomas.[15] These reports supported the hypothesis that a better prognosis of HMF might be due to the cytotoxic molecules-bearing cells.

In this report, we presented a case of HMF with dense infiltration of TILs that bear several cytotoxic molecules. In addition, our immunohistochemical study revealed that the ratio of $Foxp3^+$ Tregs is decreased in the lesional skin of HMF compared with that of conventional MF. Our case suggested possible mechanisms for the hypopigmentation and good prognosis of this type of MF.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.dsi.2015.08.006.